ELSEVIER

Archives of Medical Research 39 (2008) 52–60

Archives of Medical Research

ORIGINAL ARTICLE

Chemical Hypoxia-induced Glucose Transporter-4 Translocation in Neonatal Rat Cardiomyocytes

Fu Guan,* Bo Yu,* Guo-xian Qi, Jian Hu, Ding-yin Zeng, and Jia Luo

Department of Cardiology, The First Affiliated Hospital, China Medical University, Shenyang, China

Received for publication March 29, 2007; accepted June 25, 2007 (ARCMED-D-07-00135).

Background. AMP-activated protein kinase (AMPK) activation plays an essential role in glucose metabolism of the heart. This study aimed at investigating whether AMPK was involved in glucose transporter-4 (GLUT-4) translocation induced by azide-induced chemical hypoxia in primary cultured neonatal rat cardiomyocytes.

Methods. With or without adenine 9-β-D-arabinofuranoside (ara A, AMPK inbibitor) preincubation, primary cultured rat cardiomyocytes were randomized to several groups as incubated with azide (the respiratory chain inhibitor), insulin, or 5-aminoimidazole-4-carboxyamide-1-β-D-ribofuranoside (AICAR, an AMPK activator). Glucose uptake was measured through γ-scintillation and GLUT-4 protein was detected by Western blot for each group.

Results. Azide-induced chemical hypoxia and AICAR both increased glucose uptake and GLUT-4 translocation in cardiomyocytes, and AICAR had an additive effect on insulin action. Ara A decreased AICAR- and azide-induced glucose uptake and GLUT-4 translocation but did not affect basal or insulin-stimulated glucose uptake.

Conclusions. Azide-induced chemical hypoxia increased glucose uptake and GLUT-4 translocation in neonatal rat cardiomyocytes through a mechanism that at least was partially mediated by AMPK activation.

Key Words: Cardiomyocyte, Hypoxia, Glucose transporter-4, AMP-activated protein kinase, Azide.

Introduction

Glucose is the major metabolic substrate in the heart during ischemia/hypoxia and is transported into cardiomyocytes by members of facilitative glucose transporters (GLUTs). The most abundant glucose transporter in heart is glucose transporter 4 (GLUT-4), which translocates to the plasma membrane in response to insulin, muscle contraction or ischemia (1). It has been illustrated that insulin increases glucose uptake through GLUT-4 translocation via a pathway mediated by phosphatidyl inositol 3-kinase (PI3K) (2,3). In contrast, myocardial glucose uptake during ischemia is stimulated by GLUT-4 translocation to the cell surface through a PI3K-independent pathway (4). AMPK is activated by increased intracellular AMP and decreased creatine phosphate and is thought to be a metabolic stress protein (5). It has been demonstrated that 5-aminoimidazole-4-carboxyamide-1-β-D-ribofuranoside (AICAR), an analog of adenosine, activates AMPK through its 5′-phosphorylation product 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranosyl-5′-monophosphate (ZMP). However, a precursor of ara-ATP, adenine 9-β-D-arabinofuranoside (ara A) can inhibit AMPK activation (6). It is strongly recommended that AMPK is of vital importance for glucose metabolism in heart and may be involved in the signaling pathway of GLUT-4 translocation. Meanwhile, it has been illustrated that low dosage of azide incubation could induce a successful chemical hypoxia model of primary cultured neonatal rat cardiomyocytes (7). Thus, in this study we investigated the effect of AICAR and ara A on glucose uptake and GLUT-4 translocation in neonatal rat cardiomyocytes in order to elucidate the mechanism of hypoxia-induced GLUT-4 translocation.

*These authors contributed equally to the work.

Published previously online October 16, 2007.

Address reprint requests to: Bo Yu, Department of Cardiology, The First Affiliated Hospital, China Medical University, Nanjingbei Road 155, Shenyang, 110001, P. R. China; E-mail: cmu.yubo111@yahoo.com.cn

0188-4409/08 $–see front matter.
doi: 10.1016/j.arcmed.2007.06.022

Materials and Methods

Cell Culture

Primary cultures of cardiomyocytes were prepared from 1- to 2-day-old Wistar rats (provided by the Experimental Animal Center of China Medical University). Isolated cardiac ventricles were minced and sequentially digested in phosphate-buffered saline (PBS) containing 0.8% trypsin and 0.05% collagenase II (Gibco, Grand Island, NY) at 37°C for 10 min. This digestion exposure was repeated two to three times in turn. Cells obtained from each digestion exposure were suspended in Dulbecco's Modified Eagle Medium (DMEM) (Gibco) containing 10% fetal bovine serum (FBS) (Sigma, St. Louis, MO) and centrifuged for 10 min at 100 × g (Himac CF7D2; Hitachi, Tokyo, Japan). Pellets were resuspended and combined in the culture medium and precultured on culture dishes for 2 h to deplete fibroblasts (37°C, 5% CO_2, 95% air, NuAire 8000 Autoflow CO_2 Incubator; Thermo Electron Corporation, Franklin, MA). The resulting suspension was collected and seeded on culture dishes at a density of 1.0×10^5 cells/mL. Cells were used for experiment after 5-day culture. About 90% of cells were identified as cardiomyocytes immunocytochemically stained with monoclonal antisarcomeric actin clone 5C5 (Sigma).

Azide-induced Chemical Hypoxia

According to a previous study performed by Chen (7), sodium azide (NaN_3) was prepared fresh in culture medium for 2 h in each experiment, and cardiomyocytes were incubated with 0−10 mM azide (2 mL/dish) after 72 h cultivation (37°C, 5% CO_2, 95% air). Within 12 h, at each time point cell viability was detected by methyl thiazolyl tetrazolium (MTT) assay (*in vitro* MTT-based assay kit; Sigma) and annexin V-FITC/propidium iodide (PI) staining (apoptosis detection kit; Beijing Biosea Biotechnology Co. Ltd., Beijing, China). It is noted that wash-off experiment (namely, the azide was eliminated after incubation, and cells were incubated with normal culture medium for at least another 3 h) was used to determine the effect of azide concentration and incubation duration on cell viability. Staining was performed according to manufacturer's instructions. Five thousand cells per sample were analyzed using flow cytometer (Becton Dickinson FACSAria, Franklin Lakes, NJ).

Grouping

With/without ara A (200 μM) (+/−) preincubation, cells were randomized to several groups as basal group (C+/−) and five treated groups that were incubated with 1 mM sodium azide for 3 h (T1+/−), 500 μM AICAR for 15 min (T2+/−), 100 nM insulin for 30 min (T3+/−) or 1 mM sodium azide for 2 h and 45 min and additional 500 μM AICAR for another 15 min (T4+/−) or 100 nM insulin for 15 min and additional 500 μM AICAR for another 15 min (T5+/−). Samples of each group were prepared for measurement of 2-(^{3}H) deoxyglucose uptake. Protein concentration was determined using the Bradford reagent (Sigma) to normalize counts. The remaining were left for Western blot analysis.

2-(^{3}H)Deoxyglucose Uptake

Samples of 2 ml cell suspension (1.0×10^5 cells/mL) were used for 2-(^{3}H)deoxyglucose uptake determination in each experiment. 2-(^{3}H)deoxyglucose (1 μCi/mL) (Beijing Atom Hightech Co. Ltd., Beijing, China) was present for the estimation of glucose uptake rate under each stimulant in the presence of (U−C^{14})-mannitol (0.1 μCi/mL) (Beijing Atom Hightech Co. Ltd.) (8). Glucose uptake (pmol/mg protein/10 min) was performed for 15 min via 2-(^{3}H)deoxyglucose accumulation through γ-scintillation (Beckman LS3801 version-3.0-D). Reactions were terminated by washing cells with ice-cold phloretin buffer, a specific inhibitor of glucose transport. Protein concentration was determined using the Bradford reagent (Sigma) to normalize counts. Sample number is 3 for each group and each test was replicated three times.

Western Blotting

After treatment, cells were washed twice in ice-cold PBS and removed from the cell culture dishes by trypsinization. Cells were centrifuged at 600 × g for 5 min and then homogenized in buffer A with protease inhibitors (cell fractionation kit; Biovision, Mountain View, CA). For subcellular fractions preparation, an S1 fraction was prepared by centrifugation at 16,000 × g for 15 min. One to 2 mg of S1 protein were layered on a 5−25% glycerol gradient formed over a 50% sucrose pad and centrifuged at 60,000 × g for 70 min (9), and 0.300 mL subcellular fractions were collected. For preparation of plasma membrane-enriched fractions, homogenates were centrifuged at 200 × g for 5 min. The supernatant was removed, and plasma membranes containing fractions were pelleted by centrifugation at 16,000 × g for 15 min. This fraction contained 95% of the α_1-subunit of Na^+-K^+-ATPase, a plasma membrane marker (8). Each protein sample (20 μg) was loaded onto SDS-PAGE and transferred to PVDF membrane. Membranes were blocked overnight with a 5% (w/v) nonfat milk solution containing 10 mM Tris-Cl, pH 8.0, 150 mM NaCl, and 0.1% Tween 20 (TBST) and incubated with primary antibody for 2 h (sheep anti-GLUT-4 with 1/800 dilution; Chemicon, Temecula, CA) at 37°C. The membranes were washed again in TBST with 5% nonfat dry milk and probed with horseradish peroxidase coupled to anti-goat IgG (Sigma) for 1 h, and unbound antibody was removed by washing. Labeled proteins were detected with an enhanced chemiluminescence detection kit (ECL, Santa Cruz, CA) and exposed to radiographic film as described by the